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IN RE APPLICATION OF: Carl Sidonius, Maria ANDELA et al.

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FOR: GRANULATES CONTAINING FEED-ENZYMES

**REQUEST FOR PRIORITY UNDER 35 U.S.C. 119**  
**AND THE INTERNATIONAL CONVENTION**

Commissioner for Patents  
Alexandria, Virginia 22313

Sir:

In the matter of the above-identified application for patent, notice is hereby given that the applicant claims as priority:

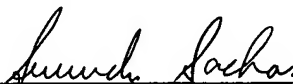
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**APPLICATION NO**  
02075017.0

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15 January 2002

Certified copies of the corresponding Convention application(s) were submitted to the International Bureau in PCT Application No. PCT/EP03/00341. Receipt of the certified copy(s) by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304.

Respectfully submitted,  
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<u>Priority date</u>	<u>Priority application No.</u>	<u>Country or regional Office or PCT receiving Office</u>	<u>Date of receipt of priority document</u>
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The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland  Facsimile No. (41-22) 338.89.70	Authorized officer  Gabriela VALENTE (Fax 338 8970)  Telephone No. (41-22) 338 8244
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Patentanmeldung Nr. Patent application No. Demande de brevet n°

02075017.0

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:  
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If no title is shown please refer to the description.  
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Granulates containing feed enzymes

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)  
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**GRANULATES CONTAINING FEED-ENZYMES**

5

**Field of the invention**

The present invention relates to the formulation of enzymes, preferably feed-enzymes, into granulates. These (edible) granulates can then be used in animal feed.

10

**Background of the invention**

Animal feed represents one of the largest costs incurred in keeping livestock and other animals. The use of various enzymes in animal, e.g. livestock, feed has become almost common practice. These enzymes are usually produced by culturing micro-organisms in large scale fermenters operated by industrial enzyme producers. At the end of the fermentation, the resulting "broth" is usually subjected to a series of filtration steps to separate the biomass (the micro-organisms) from the desired enzyme (in solution). Subsequently the enzyme solution is concentrated and processed as a liquid (often after addition of various stabilisers) or to a dry formulation.

For economical and practical reasons, dry enzyme formulations are often preferred to liquid enzymes formulations. Nevertheless, even when a dry enzyme formulation is chosen, some steps of the feed pelleting proces, such as conditioning, may be detrimental to the enzyme. Various enzyme manufacturers have developed alternative formulation methods to improve the stability of dry enzyme products during pelleting and storage of the feed. For instance, an option is to coat the enzyme-containing granule with a suitable coating agent.

EP 0 569 468 refers to a formulation consisting of an enzyme-containing granulate that is coated with a high melting wax or fat alleged to improve resistance to pelleting conditions. A disadvantage of such a coating is that the dissolution time of the granulate is long (about one hour). Therefore the bioavailability of the enzyme to the animal is decreased. In addition, the granulates have a wide particle size distribution which makes it difficult to obtain an evenly distributed enzyme concentration after coating, since small particles contain a relatively high amount of coating agent as compared to large particles.

WO 00/47060 discloses the use of polyethylene glycol (PEG) as a coating material. These PEG coatings have the disadvantage that they do not increase the pelleting stability of the enzyme granulate to the desired level.

There is thus still a need for stable formulations of enzymes for use in animal feed that are cheap and easy to produce and that combine a satisfactory pelleting stability with good bioavailability of the enzyme to the animal.

### **Detailed description of the invention**

10

The present invention discloses a process for the preparation of an enzyme-containing granulate suitable for use in an animal feed, the process comprising processing an enzyme, a solid carrier, optionally additives and water in appropriate relative amounts to obtain enzyme-containing granules, drying the granules, and coating the dried granules with a dispersion comprising particles of a hydrophobic substance dispersed in a suitable solvent. The coating of an enzyme granulate with such a dispersion provides for a low water uptake during the conditioning step of the feed preparation process and, surprisingly, an attractively short dissolution time of the enzyme granule.

In a preferred embodiment of the invention, the size of the particles in the dispersion should be sufficiently small to enable the formation of a homogeneous coating layer upon drying of the coated granules. More preferably, the particles in the dispersion should have a size between 10 and 1000 nm, most preferably between 10 and 500 nm.

The hydrophobic substance used in the dispersion for coating an enzyme granulate according to the invention preferably has a melting point which is sufficiently high to prevent melting of the hydrophobic substance coating layer under feed pellet preparation conditions. More preferably, the melting point of the hydrophobic substance is between 40 and 200 °C, most preferably between 50 and 180 °C.

It is further preferred that the hydrophobic substance dispersion is capable of forming an insoluble layer upon drying at a relatively low temperature, typically a temperature substantially below the melting point of the hydrophobic substance.

A suitable solvent to disperse the hydrophobic substance particles is a solvent wherein the hydrophobic substance is insoluble, e.g. a hydrophilic solvent like water or ethanol. Preferably, water is used as a solvent. The dispersion typically may contain 10 to

60 % hydrophobic substance in solvent, preferably 20 to 40 % hydrophobic substance in solvent.

To keep a stable dispersion, stabilising agents like surfactants may be added to the dispersion.

5        The hydrophobic substance may be a hydrophobic fat- or wax-like substance, such as a triglyceryl, diglyceryl or monoglyceryl ester of a higher fatty acid, a higher fatty alcohol, a higher fatty acid, or may be a hydrophobic polymeric compound.

10        The hydrophobic polymeric compound preferably is a polyolefin, more preferably a polyolefin wherein the olefin (hydrocarbon) monomers have a length of 2 to 10 carbon atoms, most preferably a polyolefin wherein the olefin monomers have a length of 2 to 4 carbon atoms wherein it is envisaged to optionally include monomers with a chain length of 5 to 10 carbon atoms in the polymerisation process, to obtain a polymer with short-chain branches.

15        In a preferred embodiment, the polyolefin which is used as hydrophobic polymer in the dispersion according to the invention is selected from the group consisting of polyethylene, polypropylene, polybutylene and/or polybutadiene. More preferably, the polyolefin is polyethylene and/or polypropylene.

20        The polyolefin may be a substantially linear polymer, i.e. a polymer which is not branched or only displays a low degree of branching. A substantially linear polymer thus may include a linear polymer with short side chains, i.e. with a length of at the most around 10 carbon atoms.

For polyethylene, the molecular weight may be in the range of 3000 – 20,000 Da.

25        In a preferred embodiment of the invention, the polyolefin dispersion is a dispersion of polyolefins containing acidic groups which are neutralised with an amine. The acidic groups preferably are carboxylic groups and the amine preferably is ammonia.

Carboxylic groups may conveniently be introduced into the polyolefin by, for instance, oxidation. The amount of acidic groups introduced in the polyolefin is characterised by the so-called acid number. The acid number of the polyolefin may range between 4 and 30, preferably between 12 and 18, in the case of polyethylene.

30        Additional compounds may be added to the polyolefin dispersion to confer favourable characteristics to the dispersion. For instance, the polyolefin particles may be physically stabilised with specific compounds to prevent clotting. The dispersion may also contain compounds which promote the formation of an insoluble film upon drying. Examples of additional compounds are surfactants or amines.

The hydrophobic substance coating preferably is applied at 0.1 - 20%, more preferably at 0.2 - 10% and most preferably at 0.4 - 5% of the weight of the granules. The percentages used throughout this specification refer to weight percentages and, unless indicated otherwise, are based on the weight of the dry granulate, before coating.

5 To apply the hydrophobic substance and, optionally, other coating material(s) onto the granulate a number of known methods are available which include the use of a fluidised bed, a high shear granulator, a mixer granulator, or a Nauta-type of mixer. In a preferred method for application of the polyethylene onto the granulate, the polyethylene is sprayed as a dispersion onto a fluidized bed of the granules to be coated.

10 The granule to be coated according to the present invention comprises a feed enzyme, a solid carrier and, optionally, one or more additives.

The solid carrier to be used in the preparation of the granulate is a powder which can be compacted into a granule and which preferably has an average particle size ranging between 5 and 20  $\mu\text{m}$ .

15 In a preferred embodiment of the present invention, the solid carrier essentially consists of an edible carbohydrate polymer. The numerous advantages of the use of an edible carbohydrate polymer are presented in patent application WO 98/54980.

An edible carbohydrate polymer is a carbohydrate polymer which is allowed to be used as a feed additive. The edible carbohydrate polymer is chosen so that it is edible by  
20 the animal for whom the feed is intended, and preferably digestible as well. The polymer preferably comprises hexose polymer units, more preferably glucose polymer units. Most preferably the carbohydrate polymer comprises  $\alpha$ -D-glucopyranose units, amylose (a linear (1 $\rightarrow$ 4)  $\alpha$ -D-glucan polymer) and/or amylopectin (a branched D-glucan with  $\alpha$ -D-(1 $\rightarrow$ 4) and  $\alpha$ -D-(1 $\rightarrow$ 6) linkages). Starch is the preferred carbohydrate polymer. Other  
25 suitable hexose-containing polymers that can be used instead of, or in addition to starch, include  $\alpha$ -glucans,  $\beta$ -glucans, pectin (such as proto-pectin), and glycogen. Derivatives of these carbohydrate polymers, such as ethers and/or esters thereof, are also contemplated. Gelatinised starch is best avoided and thus may not be present. Suitably the carbohydrate polymer is water-insoluble.

30 In the examples described herein corn-, potato- and rice-starch is used. However, starch obtained from other (e.g. plant, such as vegetable or crop) sources such as tapioca, cassava, wheat, maize, sago, rye, oat, barley, yam, sorghum, or arrowroot is equally applicable. Similarly both native as well as modified (e.g. dextrin) types of starch can be used in the invention. Preferably the carbohydrate (e.g. starch) contains little or no protein,



preferably less than 5% (w/w), more preferably less than 2% (w/w), most preferably less than 1% (w/w).

In other embodiments of the invention, one or more additional ingredients may be incorporated into the granulate, e.g. as processing aids and/or for further improvement of the pelleting stability and/or the storage stability of the granulate. A number of such additives are discussed below.

In one embodiment of the invention, the additive comprises a water soluble inorganic salt (as suggested in EP 0 758 018). Preferably, the granules comprise at least 0.1% of a water soluble inorganic salt comprising a divalent cation, more preferably zinc. Most preferably, the inorganic salt is zinc-sulphate. The end product preferably contains from 500 - 1,500 mg Zn/kg end product, more preferably 700 - 1,300 mg Zn/kg end product and most preferably 900 - 1,100 mg Zn/kg end product. Divalent cations are preferred because they provide the best storage and processing stability. Sulphate is preferred as anion because it provides the best drying yield. The salts may be added (e.g. to the mixture) in solid form. Alternatively, the salt(s) may be dissolved in the water or enzyme-containing liquid prior to mixing with the solid carrier.

Further improvement of the pelleting stability may be obtained by the incorporation of hydrophobic, gel-forming and/or slowly dissolving compounds into the formulation. These may be provided by adding at least 0.1% (w/w), preferably at least 0.5% and more preferably at least 1% of the desired compound (based on the weight of water and solid carrier ingredients) to the mixture to be processed to granules. Suitable substances include derivatised celluloses, such as HPMC (hydroxy-propyl-methyl-cellulose), CMC (carboxy-methyl-cellulose), HEC (hydroxy-ethyl-cellulose), polyvinyl alcohols (PVA); and/or edible oils. Edible oils, such as soy oil or canola oil, may be added (e.g. to the mixture to be granulated) as a processing aid.

In the process of preparing the granulate, the enzyme and water are preferably provided as an enzyme-containing (preferably aqueous) liquid, such as a solution or a slurry, that is from, or derived from, a microbial fermentation process. This fermentation process will usually be one in which the enzyme is produced. The fermentation process may result in a broth which contains the micro-organisms (which produced the desired enzyme) and an aqueous solution. This aqueous solution, once separated from the micro-organisms (for example, by filtration) can be the enzyme-containing aqueous liquid used in the invention. Thus in preferred embodiments the enzyme-containing aqueous liquid is a filtrate. Usually the enzyme will be in an active form. Preferably the liquid is in a

concentrated form, such as an ultra-filtrate (UF), which may allow the production of a granulate with a desired activity level.

The amount of enzyme-containing liquid (and so enzyme) that can be absorbed onto the carrier is usually limited by the amount of water that can be absorbed. The enzyme solution may contain about 25% (w/w) dry matter. The amount of water added to the solid carrier is such that (substantially) all the water in the aqueous liquid is absorbed by all the components present in the solid carrier. The use of higher temperatures in order to absorb a greater amount enzyme-containing liquid is also contemplated by the present invention, and indeed is preferable especially when dealing with thermostable enzymes. For these enzymes therefore mixing of the solid carrier and liquid (or enzyme and water) is performed at a temperature above 30°C, preferably above 40°C and more preferably above 50°C. Alternatively or in addition, the liquid may be provided at this temperature. In general, non-swelling conditions of the solid carrier (at lower temperatures) are preferred to minimise loss arising from instability of (heat sensitive) enzymes at higher temperatures.

The water or enzyme-containing liquid may comprise one or more enzyme(s). Suitable enzyme(s) are feed enzymes to be included in animal feed (including pet food). The function of these feed enzymes is often to improve the feed conversion rate, e.g. by reducing the viscosity or by reducing the anti-nutritional effect of certain feed compounds. Feed enzymes (such as phytase) may also be used such as to reduce the amount of compounds in the manure which are harmful to the environment.

In a preferred embodiment, granules are prepared according to the process of the invention which contain a high concentration of a feed enzyme. The advantages of preparing phytase compositions at a high concentration was already described in WO 98/55599.

Suitable enzymes include: phosphatases, such as phytases (both 3-phytases and 6-phytases) and/or acid phosphatases, carbohydrases, such as amylolytic enzymes and/or plant cell wall degrading enzymes including cellulases such as  $\beta$ -glucanases and/or hemicellulases such as xylanases or galactanases, proteases or peptidases such as lysozyme, galactosidases, pectinases, esterases, lipases, phospholipases such as the mammalian pancreatic phospholipases A2 and glucose oxidase. Preferably, feed enzymes have a neutral and/or acidic pH optimum. More preferably, the feed enzyme at least is an enzyme selected from the group consisting of phytases, xylanases,  $\beta$ -glucanases, phospholipases and glucose oxidases. Most preferably, the feed enzyme at least is an enzyme selected from the group consisting of phytases and xylanases.

If the enzyme is a phytase, then the final granulate may preferably have an enzyme activity ranging from 4,000 to 20,000 FTU/g, more preferably from 5,000 to 20,000 FTU/g, most preferably from 5,000 to 15,000 FTU/g. One Phytase Unit (FTU) is thereby defined as the amount of enzyme which liberates 1  $\mu$ mol inorganic phosphate per minute from sodium phytate (0.0051 mol/litre) at 37°C and at a pH of 5.5 under the conditions that phytase activity was determined according to the procedure "ISL-method 61696" (manual molybdate-vanadate assay).

If the enzyme is a xylanase, then the final granulate may preferably have an enzyme activity ranging from 5,000 to 100,000 EXU/g, more preferably from 10,000 to 100,000 EXU/g, and most preferably from 15,000 to 100,000 EXU/g. One Endo-Xylanase Unit (EXU) is thereby defined as the amount of enzyme which liberates 4.53  $\mu$ mol reducing sugars, measured as xylose equivalents, per minute under the conditions of the procedure "ISL-method 61731".

ISL-methods are obtainable on request from DSM, Food Specialties, Agri Ingredients, Wateringseweg 1, P.O. Box 1, 2600 MA, Delft, The Netherlands.

In addition to these feed enzymes, the invention is equally applicable to non-enzymatic polypeptides with biological activities, such as antigenic determinants to be used as vaccines and/or polypeptides engineered to have an increased content of essential amino acids, of which the biological activity may be sensitive to thermal inactivation.

The mechanical processing used in the present invention for making the mixture of the enzyme, water (e.g. an enzyme-containing liquid), a solid carrier and optionally additives can be known techniques frequently used in food, feed and enzyme formulation processes. This mechanical processing for instance comprises expansion, extrusion, spheronisation, pelleting, high-shear granulation, drum granulation, fluid bed agglomeration or a combination thereof. These processes are usually characterised by an input of mechanical energy, such as the rotation of a screw or a mixing mechanism, the pressure of a rolling mechanism of a pelleting apparatus, the movement of particles by a rotating bottom plate of a fluid bed agglomerator or the movement of the particles by a gas stream, or a combination thereof. These processes allow the solid carrier (e.g. in the form of a powder), to be mixed with the enzyme and water, for example an enzyme-containing liquid (an aqueous solution or slurry), and so subsequently granulated. Alternatively the solid carrier can be mixed with the enzyme (e.g. in a powder form) to which water, such as a liquid (or slurry) is then added (which can act as granulating liquid).

In yet a further embodiment of the invention the granulate (e.g. an agglomerate) is formed by spraying or coating the enzyme-containing liquid onto a carrier, such as in a fluid bed agglomerator. Here the resulting granules can include an agglomerate as can be produced in a fluid bed agglomerator. Preferably the mixing of the enzyme-containing liquid and the solid carrier additionally comprises kneading of the mixture before extrusion.  
5 This may improve the plasticity of the mixture in order to facilitate granulation.

If the granulate is formed by extrusion this is preferably performed at low pressure. This may offer the advantage that the temperature of the mixture being extruded will not, or only slightly, increase. Low-pressure extrusion includes extrusion for example in a Fuji  
10 Paudal-type of basket- or dome- extruder.

The extrudate obtained can be subjected to rounding off (e.g. spheronisation), such as in a Marumeriser™ and/or compaction. The extrudate can be spheronised prior to drying since this may reduce dust formation in the final granulate and/or may facilitate any coating of the granulate.

15 The granules can then be dried, such as in a fluid bed drier or, in case of the fluid bed agglomeration, can be immediately dried (in the agglomerator) to obtain (solid dry) granulates. Other known methods for drying granules in the food, feed or enzyme industry can be used by the skilled person. Suitably the granulate is flowable. The drying preferably takes place at a product temperature from 25 to 60°C, preferably from 30 to 50°C.

20 Preferably the granules have a relatively narrow size distribution (e.g. they are monodisperse). This can facilitate a homogeneous distribution of the enzyme granulate in the feed pellets. The process of the invention tends to produce granulates with a narrow size distribution. The size distribution of the granulate is suitably between 100 µm and 2,000 µm, preferably between 200 µm and 1,800 µm, more preferably between 400 µm  
25 and 1,600 µm and most preferably between 500 and 1,000 µm. The granules may be of irregular (but preferably regular) shape, for example approximately spherical.

If necessary, an additional step may be included in the process to further narrow the size distribution of the granules, such as screening. For instance, this additional screening step will select granules having a size distribution between 0.5 and 1 mm.

30 The enzyme-containing granulate obtainable by these processes (which forms another aspect of the invention) seeks to solve or at least mitigate the problems encountered in the prior art. These coated granulates have a high pelleting stability and a short dissolution time. Therefore the bioavailability of the enzyme to the animal is improved as compared to classical fat-coated granules.

The invention thus provides an enzyme-containing granulate obtainable by the above-mentioned processes and having the following characteristics. The granulate consists of granules containing a feed enzyme, a solid carrier and, optionally, one or more additives, coated with a polyolefin. Preferably, said additives comprise at least one of a gel-forming or slow dissolving compound such as polyvinyl alcohol and a water soluble inorganic salt.

The granulate of the invention is suitable for use in the preparation of an animal feed. In such processes the granulate is mixed with feed substances, as such, as part of a premix or as precursor to an animal feed. The characteristics of the granulate according to the invention allows its use as a component of a mixture which is well suited as an animal feed, especially if the mixture is steam treated, subsequently pelleted and optionally dried.

Thus, a further aspect of the present invention relates to a process for the preparation of animal feed, or a premix or precursor to an animal feed, the process comprising mixing the granulate provided by the present invention with one or more animal feed substances or ingredients.

The present invention also relates to a process for promoting the growth of an animal, the process comprising feeding an animal a diet that comprises the granulate provided by the invention. Here, the animal diet can include either the granulate itself, or the granulate present in a feed. Suitable animals include farm animals, such as livestock, pigs and poultry.

Another aspect of the invention thus relates to a composition comprising the granulate of the invention, which composition is preferably an edible feed composition such as an animal feed.

Still another aspect of the present invention relates to the use of the granulate of the invention in, or as a component of, an animal feed or for use in an animal diet.

Preferred features and characteristics of one aspect of the invention are equally applicable to another mutatis mutandis.

The following Examples are presented merely to illustrate the invention, and are not intended, or to be construed as, being limiting.

## **EXAMPLES**

### **General methods**

### Preparation of phytase-containing granules

Phytase-containing granules are prepared according to the process described in WO98/54980.

5

### Conditioning/pelleting step:

50 grams granules were mixed in 10 kg feed of the premix of choice and mixed just before the test with 240 kg of the same recipe. This 250 kg mixture was dosed in a mixer/conditioner by a dosing screw, at a speed of 600 kg/h, where it was heated by direct steam around 80°C. The residence time was about 30-40 seconds whereupon the hot mixture was pushed into the pelleting press. The pellets coming out of the die have a temperature between 80 and 82°C and fall on a cooling belt. From this belt, samples were taken for the stability measurement.

15

### Poultry feed used for analysis of pelleting stability:

Maize (50%), peas (5%), soybean meal (28%), tapioca (1.98%), fish meal (2.5%), feather meal (1.5%), soybean oil (1.75%), animal fat (3.5%), Mervit (Premervo, Utrecht, The Netherlands) 100 (1%), limestone (1.1%), mono calcium phosphate (1.22%), salt (0.3%), Mervit 394 (0.65%), Mervit 393 (1.5%), up to a total of 100%.

20

### Analysis of phytase activity in feed pellets:

Phytase activity was determined according to the procedure "ISL-method 61696" (manual molybdate-vanadate assay).

25

### Example 1

300 grams uncoated granules (batch NPHG 498) were put into a STREA fluid bed apparatus from NIRO-AEROMATIC.

With a topspray device (two-phase nozzle), various coatings were sprayed onto the granules as indicated in Table 1. These coated granules were tested in the pelleting test as described under General methods.

30

HS granules are granules coated with a fat + anti-caking coating (>30%) (obtained from Novo Nordisk, batch no. HF 98011450).

**Table 1:** Residual phytase activity in poultry feed in % after conditioning/pelleting at 80/81°C.

Sample no. with coating	Dissolution time (min.)	Residual activity (%)
A uncoated batch 498	1	27
B 10% PEG 6000 solution	2	30
C 10% Carnauba wax dispersion	1	36
D 10% PE dispersion Stamylan® LD1965	1	32
E 10% PE dispersion EXACT® 8210	20	48
F 8% PE Dispersion 1205	5	57
G HS granule	>60	55

5 Surprisingly, the dissolution time of the granulates does not seem to correlate with their pelleting stability. The most important parameter to optimise is the dissolution time, since it determines the bioavailability of the enzyme to the animal.

The coatings of samples C and F were bought from Paramelt B.V. (Heerhugowaard, The Netherlands) and of samples D and E from DSM Research (DSM N.V., Geleen, The Netherlands).

### Example 2

The effect of the coating Dispersion 1205 on the pelleting stability of phytase-containing granulates was tested in large scale production.

5 In a GPCG 300 fluid bed machine from Glatt, 300 kg of uncoated granulate, prepared as described under General methods, was coated with 120 kg Dispersion 1205 à 19.5% dry matter (sample L) and another batch with 60 kg PEG 6000 solution à 50% dry matter (sample K).

10 These coated granulates were tested in a pelleting test together with the uncoated product (sample H) and HS granule of a competitor (sample M) as described under General methods.

The results are presented in Table 2.

15 **Table 2:** Residual phytase activity in poultry feed in % after conditioning/pelleting at 80/80°C.

Sample no. with coating	Dissolution time (min.)	Residual activity (%)
H Uncoated batch S4893	1	33
K Coated with PEG 6000	2	36
L Coated with PE Dispersion 1205	10	74
M HS-granule	>60	76

### Example 3

20 In a Glatt GPCG 1.1 fluid bed coater, 1 kg of uncoated granulate, prepared as described under General methods, was coated with different coatings as mentioned in Table 3.

Samples O was coated with a melt at a temperature of 80°C. Sample P was coated with a self-made dispersion of sodium caseinate and hydrogenated coconut fat.



**Table 3: Residual phytase activity in poultry feed in % after conditioning/pelleting at 80/80°C.**

Sample no. with coating	Dissolution time (min.)	Residual activity (%)
N Uncoated batch 5193	1	20
O Coated with 10% Waretta fat	4	22
P Coated with 10% fat dispersion	10	25
M HS granule	>60	53

- 5 The various fat-type coatings tested (except the one from the HS granule M) confer a short dissolution time to the granules, but the pelleting stability of the coated granules is not significantly improved as compared to the uncoated granulate (sample N).

#### Example 4

- 10 The influence of different concentrations of Dispersion 1205 (2 to 24%, samples S to V) on the pelleting stability and on the dissolution time of the granulate was tested. Uncoated granulates were prepared as described under General methods. The granules were coated as indicated in Table 4 and tested in a pelleting test as described under General Methods.

- 15 The results are presented in Table 4.

**Table 4: Residual phytase activity in poultry feed in % after conditioning/pelleting at 80/80°C.**

Sample no. with coating	Dissolution time (min.)	Residual activity (%)
R Uncoated batch 5193	1	22
S Coated with 2% Dispersion1205	2	31
T Coated with 4% Dispersion 1205	2	49
U Coated with 8% Dispersion 1205	2	58
V Coated with 24% Dispersion 1205	60	75
M HS granule	>60	64

- 20 The concentration of the polyethylene which is applied to coat the granules seems to be positively correlated with the dissolution time of the granules.

### Example 5

In this example the influence of a polypropylene (PP) dispersion and of polyethylene (PE) dispersions from different suppliers were tested. Uncoated granules were prepared as described General methods. The granules were then coated as indicated in Table 5 and tested in a pelleting test as described under general methods.

The results are presented in Table 5.

**Table 5:** Residual phytase activity in poultry feed in % after conditioning/pelleting at 80/80°C.

Sample no. with coating	Dissolution time (min)	Residual activity (%)
W Uncoated batch 5193	1	21
X Coated with 4% Permanol Z (PE)	1	38
Y Coated with 4% Permanol 601 (PP)	1	43
Z Coated with Dispersion 1205 (PE)	1	39

All three coatings, the Permanol samples (X+Y) from Clariant GMBH, Frankfurt am Main) and the Dispersion 1205 from Paramelt BV gave about a twice as high residual activity than the uncoated sample and still ensured the same dissolution time.

The Permanol 601 is a PP coating with a higher melting point (30°C higher) than the PE, but also with a larger particle size (10 times larger) in the dispersion than the Dispersion 1205.

### CLAIMS

1. A process for the preparation of an enzyme-containing granulate suitable for use in an animal feed, the process comprising processing a feed enzyme, a solid carrier,  
5 optionally additives and water in appropriate relative amounts to obtain enzyme-containing granules, drying the granules and coating the dried granules with a dispersion of particles of a hydrophobic substance dispersed in a suitable solvent.
2. A process according to claim 1, wherein the particles have a size between 10 and  
10 1000 nm, preferably between 10 and 500 nm.
3. A process according to claim 1 or 2, wherein the hydrophobic substance has a melting point between 40 and 200 °C, preferably between 50 and 180 °C.
- 15 4. A process according to any one of claims 1 - 3, wherein the hydrophobic substance is applied at 0.1 - 20%, preferably at 0.2 - 10%, more preferably at 0.4 - 5%.
5. A process according to any one of claims 1 - 4, wherein the solvent is water.
- 20 6. A process according to any one of claims 1 - 5, wherein the hydrophobic substance dispersion contains 10 to 60 % (w/w) hydrophobic substance, preferably 20 to 40 % (w/w) hydrophobic substance.
7. A process according to any one of claims 1 - 6, wherein the hydrophobic substance  
25 is a hydrophobic fat- or wax-like substance and/or a hydrophobic polymer.
8. A process according to claim 7, wherein the hydrophobic polymer is a polyolefin.
9. A process according to claim 8, wherein the polyolefin is polyethylene and/or  
30 polypropylene.
10. A process according to claim 8 or 9, wherein the polyolefin dispersion is a dispersion of polyolefins containing acidic groups which are neutralised by an amine.

11. A process according to claim 10, wherein the acidic groups are carboxylic groups.

12. A process according to claim 10 or 11, wherein the amine is ammonia.

5 13. A process according to any one of claims 1 - 12, wherein the enzyme is a phytase, xylanase,  $\beta$ -glucanase, phospholipase, amylase and/or glucose oxidase.

14. An enzyme-containing granulate coated with a dispersion of particles consisting essentially of a hydrophobic substance obtainable by a process according to any one of  
10 claims 1 to 13.

15. A process for the preparation of an animal feed, or a premix or precursor to an animal feed, said process comprising mixing a granulate according to claim 14 with one or more animal feed substances or ingredients.

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16. The process according to claim 15, wherein the mixture of feed substance(s) and granulate is treated with steam, pelletised and cooled.

20

17. A feed-composition-comprising a granulate according to claim 14. . . . .

18. A process for promoting the growth of an animal, the process comprising feeding an animal a diet that comprises either a granulate according to claim 14 or a composition according to claim 17.

25 19. Use of a granulate according to claim 14 in an animal feed or as a component in an animal diet.

30

20. Use of a granulate according to claim 14 to improve the pelleting stability of the enzyme.

**GRANULATES CONTAINING FEED ENZYMES**ABSTRACT

5 The present invention describes a process for the preparation of an enzyme-containing granulate wherein an aqueous enzyme-containing liquid is mixed with a solid carrier and optionally additive ingredients and is mechanically processed into granules, dried and subsequently coated with a dispersion containing particles of a hydrophobic substance. The hydrophobic substance may be a hydrophobic fat- or wax-like substance or a  
10 hydrophobic polymeric compound. Preferably, the hydrophobic polymeric compound is a polyolefin, more preferably polyethylene and/or polypropylene. The thus coated enzyme granulate is suitable for the manufacture of animal feed compositions by mixing the granulate with feed ingredients, treating with steam and pelleting. The coated granulates show high enzyme stability during pelleting conditions and during storage. At the same  
15 time, the dissolution time of the granule is very short so that the bioavailability of the enzyme to the animal is improved.

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